JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1992, p. 3268–3269
0095-1137/92/123268-02$02.00/0

Vol. 30, No. 12

NOTES

Novel Coagglutination Method for Serotyping Group B Streptococci

STELLAN HÅKANSSON,[1,2]* LARS G. BURMAN,[3] JÖRGEN HENRICHSEN,[4] AND STIG E. HOLM[2]

Departments of Pediatrics[1] and Clinical Bacteriology,[2] University of Umeå, S-901 85 Umeå, and National Bacteriological Laboratory, S-105 21 Stockholm,[3] Sweden, and Department of Bacteriology, Statens Seruminstitut, DK-2300, Copenhagen S, Denmark[4]

Received 29 June 1992/Accepted 31 August 1992

A group G streptococcal strain was coated with antibody against six different serotypes (Ia, Ib, II, III, IV, and V) of group B streptococci. The coagglutination patterns of 114 strains of group B streptococci were compared with the serotypes determined after immunoprecipitation. The specificity of the method was 100% and the sensitivity 97%. It was used for the typing of 89 invasive and 101 colonizing isolates. The new method is swift, specific, and highly sensitive. It consumes only minute amounts of antibody.

Traditional methods for serotyping of group B streptococci (GBS) involve the extraction of capsular polysaccharide by HCl and subsequent precipitation of the antigen by type-specific antibody (5, 6). These methods are time-consuming and use relatively large amounts of antiserum. An alternative method, using coagglutination with the Cowan I strain of *Staphylococcus aureus*, has been described previously (2). We developed a novel coagglutination method using group G streptococci (GGS) coated with purified immunoglobulin G (IgG) against GBS type antigens. In the study described here, we compared this method of typing with the traditional immunoprecipitation method.

A GGS strain (G148; kindly supplied by Göran Kronvall, Karolinska Institute, Stockholm, Sweden) was used as the antibody carrier. This strain is rich in protein G on its surface and binds efficiently to the Fc portion of IgG from several species (1). Monospecific rabbit IgG preparations (purified by ammonium sulfate precipitation and gel filtration) against six different serotypes of GBS (Ia, Ib, II, III, IV, and V) were obtained from Dako A/S (Glostrup, Denmark).

Strain G148 was cultured overnight in ultrafiltered (molecular weight cutoff, 10,000) T-Y medium (4). A batch of 12 liters was started by the addition of a 120-ml overnight culture in the same medium. The bacteria were pelleted, washed twice with phosphate-buffered saline (PBS; pH 7.2), and inactivated by heat (60°C, 3 h). After inactivation, the cells were again washed twice as described above. The cell pellet was divided into six equal parts, and each part was resuspended in 200 ml of PBS, to which 0.3 ml of the respective antibody preparations (containing 20 mg of protein per ml) was added. This mixture was slowly agitated for 1 h at room temperature. Excess antibody was removed by washing twice in PBS, and the pellet was resuspended to a final volume of 120 ml. To this preparation, 15 mM sodium azide was added as a preservative.

For coagglutination, 50 μl of coated GGS was mixed with a fraction of a 10-μl loop of bacteria (total, approximately 3 μl) grown on blood agar plates. The mixture was gently rocked on a black glass slide. If the reaction was positive, a distinct agglutination occurred rapidly, usually within 30 s. If there was no visible agglutination after 2 min, the reaction was negative.

Sixty GBS strains, originally typed by immunoprecipitation (6) in Copenhagen, were coded and typed by coagglutination. This set of strains contained 10 strains each of serotypes Ia, II, III, and IV. There were 9 type Ib and 11 type V strains.

Fifty-four GBS strains from various sources, isolated at the Department of Clinical Bacteriology of the University Hospital of Umeå, were typed by coagglutination, coded, and sent to Copenhagen for serotyping by immunoprecipitation (the number of strains of each serotype was as follows: Ia, 19; Ib, 6; II, 7; III, 17; IV, 1; nontypeable, 4).

After this validation, a total of 190 GBS strains (89 isolates from blood or cerebrospinal fluid of neonates; 101 vaginal colonizing isolates) were typed by the coagglutination method. Strains which were nontypeable by coagglutination were retyped, together with positive controls for serotypes I to V, by immunodiffusion (5).

A total of 114 strains from various sources were typed by the new coagglutination method and were compared with the immunoprecipitation method by the method described by Lancefield (6). There was complete agreement between the results of the two methods for 110 of the strains. The serotypes of four strains could not be determined because of spontaneous agglutination of the bacteria with GGS. The spontaneous agglutination of these strains was not abrogated after trypsin treatment. Three strains were distinctly typed by immunoprecipitation, and one strain was nontypeable. Thus, the sensitivity of the coagglutination method was 96.5% and the specificity was 100%.

All 89 invasive isolates of GBS gave a distinct agglutination pattern by the new assay. None of these strains spontaneously agglutinated with GGS. Nontypeable strains were more common among the vaginal colonizing isolates. A total of 11 strains, which were all positive for the group antigen B (Streptex; Wellcome), could not be allocated to a definite

* Corresponding author.

TABLE 1. Distribution of serotypes among invasive and colonizing GBS isolates

Serotype	No. (%) of the following GBS isolates:	
	Invasive	Colonizing
Ia	13 (15)	11 (11)
Ib	11 (12)	18 (17)
II	2 (2)	8 (9)
III	61 (69)	46 (45)
IV	0 (0)	3 (3)
V	2 (2)	4 (4)
NT[a]	0 (0)	11 (11)
Total	89 (100)	101 (100)

[a] NT, nontypeable.

serotype. Three of these strains agglutinated spontaneously, whereas eight strains gave no reaction with either serotype. The nontypeable strains were retyped by immunodiffusion against HCl-extracted type antigen (5), and all of them were negative for the six tested serotypes. The distribution frequency of the different serotypes of these isolates is shown in Table 1.

The coagglutination method for serotyping GBS is swift and simple and consumes only minute amounts of antiserum. For each serotype, a batch sufficient for 2,400 typings used 0.3 ml of purified antibody, corresponding to 2.5 μg of IgG per test. The test was 100% specific when compared with the traditional immunoprecipitation method. A few strains agglutinated GGS spontaneously, and with these strains the method is not applicable. Of 304 strains, 7 strains agglutinated spontaneously, corresponding to a sensitivity of 98% when the total material of the strains is considered. However, strains that did not agglutinate at all appeared to be truly nontypeable (i.e., capsule deficient or belonging to candidate serotypes other than serotypes I to V). The serotypes of all invasive GBS isolates were readily determined. This fact probably reflects the presence of increased amounts of capsular polysaccharide in these strains (3, 7). Among the colonizing isolates, with less or nil amounts of polysaccharide (3, 7) one would anticipate an increased number of strains which are nontypeable.

We conclude that the majority of GBS strains can be serotyped by the coagglutination method. In case the GBS strains spontaneously agglutinate the GGS, traditional serotyping by immunoprecipitation of HCl-extracted antigen should be used.

REFERENCES

1. **Björk, L., and G. Kronvall.** 1984. Purification and some properties of streptococcal protein G. A novel IgG-binding reagent. J. Immunol. **133**:969–974.
2. **Finch, R. G.** 1979. Serotyping of group B streptococci by a slide coagglutination method, p. 258–259. *In* M. T. Parker (ed.), Pathogenic streptococci. Reedbooks Ltd., Chertsey, England.
3. **Håkansson, S., S. E. Holm, and M. Wagner.** 1987. Density profile of group B streptococci, type III, and its possible relation to enhanced virulence. J. Clin. Microbiol. **25**:714–718.
4. **Holm, S. E., and E. Falsen.** 1967. An antigen-free medium for cultivation of beta-hemolytic streptococci. APMIS **69**:264–276.
5. **Jelinkova, J.** 1977. Group B streptococci in the human population. Curr. Top. Microbiol. Immunol. **76**:127–165.
6. **Lancefield, R. C.** 1933. A serological differentiation of human and other groups of hemolytic streptococci. J. Exp. Med. **57**:571–595.
7. **Yeung, M. K., and S. J. Mattingly.** 1984. Biosynthetic capacity for type-specific antigen synthesis determines the virulence of serotype III of group B streptococci. Infect. Immun. **44**:217–221.